of claim 98 under conditions suitable for production of the protein; and (b) recovering the protein.

REMARKS

Claims 51-70 have been canceled. New claims 71-99 are based on claims 51-70 and are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. Objection to Claim 60

Claim 60 is objected to because the identity of the strain is unclear and the Office Action suggests amending claim 60 to read "... contained in *E. coli* pPH6 as deposited with NRRL under accession number B-30142." Applicants have cancelled claim 60, but have incorporated the Examiner's suggestion in corresponding new claim 87.

II. The Rejection of Claims 61, 66, and 67-70 under 35 U.S.C. § 112, Second Paragraph

Claims 61, 66, and 67-70 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several grounds.

Ground 1: The Office Action states that claim 61 is indefinite because it is not possible to synthesize or make a nucleic acid solely by hybridization. The Office Action suggests wording the claim to recite "A nucleic acid isolated by (a) hybridizing a DNA under low stringency conditions ..." Claim 61 has been canceled, but this suggested language has been incorporated into corresponding new claim 89.

Ground 2: The Office Action states that claim 66 is indefinite because the limitation "the host cell" lacks sufficient antecedent basis. Applicants note that claim 66 incorrectly depends on claim 65, and should have depended on claim 64. Proper dependency has been made in the new claims.

Ground 3: The Office Action states that claims 67-70 are indefinite because claim 67 recites the term "foreign" which does not provide enough information to determine the scope of the claim. This rejection is respectfully traversed. Applicants assert that one of ordinary

skill in the art would readily understand that the term "foreign" means the signal peptide encoding nucleic acid sequence is operably linked to a heterologous gene. In other words, the signal peptide nucleic acids are not found naturally in the gene to which they are operably linked.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejections.

III. The Rejection of Claims 51, 52, 54, 56, 58, 59, and 61-66 under 35 U.S.C. § 112, First Paragraph

Claims 51, 52, 54, 56, 58, 59, and 61-66 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Specifically, the Office Action states:

> The skilled artisan cannot envision the detailed chemical structure of all of the encompassed nucleic acid sequences as claimed encoding phospholipase B activity isolated from any and all microorganisms, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Although applicants describe the sequence of only one phospholipase B gene, the claim encompasses any and all sequences having only 65% amino acid identity or 65% nucleic acid homology which have phospholipase B activity. Applicants have only provided evidence for the possession of the nucleic acid of SEQ ID NO:1 and the amino acid sequence for SEQ ID NO:2. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it.

This rejection is respectfully traversed.

The instant invention is directed to isolated nucleic acid sequences encoding a polypeptide having phospholipase B activity, selected from the group consisting of: (a) a nucleic acid sequence encoding a polypeptide having an amino acid sequence which has at least 80% identity with amino acids 20 to 464 of SEQ ID NO:2; (b) a nucleic acid sequence having at least 80% homology with nucleotides 568 to 2045 of SEQ ID NO:1; (c) a nucleic acid sequence which hybridizes under medium stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO:1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO:1, or (iii) a complementary strand of (i) or (ii); and (d) a subsequence of (a), (b),

or (c), wherein the subsequence encodes a polypeptide fragment which has phospholipase B activity.

The Office Action suggests that the specification fails to describe in sufficient detail the essential elements of the phospholipase B sequences present in microorganisms other than *Aspergillus oryzae*. Applicants assert that the specification describes the claimed invention with sufficient relevant identifying characteristics, such that a person skilled in the art would recognize that Applicants had possession of the claimed invention at the time filing.

Applicants have provided a detailed written description on how to isolate and identify the nucleic acid sequences of the claimed invention. Applicants detail on page 5, line 1, to page 7, line 7, of the specification, instructions for performing standard Southern hybridization under medium, medium-high, and high stringency conditions to identify such nucleic acids from other strains, whether of the same or different genera or species. In short, the hybridization methods describe the use of specific probes in enabling detail for identifying other phospholipase genes which hybridize under medium, medium-high, or high stringency conditions with the probes. The probes described in the specification are (i) nucleotides 568 to 2045 of SEQ ID NO:1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO:1, and (iii) a complementary strand of (i) or (ii). One of ordinary skill in the art would recognize that the use of such probes under medium, medium-high, or high stringency conditions allows the identification of other phospholipase genes which are closely related or essentially identical to the gene contained in SEQ ID NO:1. For example, Applicants have provided details in Example 2 for probing a genomic DNA library of an *Aspergillus* strain.

Applicants also detail on page 3, line 25, to page 4, line 7, of the specification, instructions for determining the degree of identity between two amino acid sequences by the Clustal method (Thompson *et al.*, 1994, *Nucleic Acids Research* 22: 4673-4680; Thompson *et al.*, 1997, *Nucleic Acids Research* 25: 4876-4882), and on page 12, line 29, to page 13, line 8, of the specification, the degree of homology between two nucleic acid sequences by the Wilbur-Lipman method (Wilbur and Lipman, 1983, *Proceedings of the National Academy of Science USA* 80: 726-730). Percent identity is determined by a direct consecutive comparison of the amino acids of the polypeptide corresponding to the amino acids of a reference polypeptide, *i.e.*, the amino acid sequence of SEQ ID NO:2 of the claimed invention. It is well known in the art that a protein that has 65% identity on the amino acid level to a reference protein, e.g., SEQ ID NO:2, is closely related to the reference protein. One of ordinary skill in the art would recognize that a nucleic acid sequence encoding such a

polypeptide will have essentially the same inherent properties as the claimed reference polypeptide of SEQ ID NO:2. As the percent identity between the two proteins increases to 70%, 80%, 90%, etc., the two polypeptides having phospholipase B activity will have essentially the same inherent properties. A similar statement can also be made using percent homology as the determinant of encoded polypeptides with similar biochemical properties.

One of ordinary skill in the art would further recognize that amino acid changes of SEQ ID NO:2 of a minor nature could be made, naturally or recombinantly, that do not change the inherent properties of the polypeptide of SEQ ID NO:2. Such amino acid changes include, for example, conservative amino acid substitutions that do not significantly affect the folding and/or activity of the protein; and small deletions, typically of one to about 30 amino acids. Such conservative substitutions are, for example, within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions which do not generally alter the specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. The most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly as well as these in reverse. It would not be surprising to one skilled in the art that a protein containing, for example, 100 amino acids could easily be modified by making 5, 10, 15, 20, or more conservative substitutions without changing the inherent functional properties of the protein.

In the instant case, claims limited to the nucleic acid sequence of SEQ ID NO:1 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another nucleic acid sequence encoding a polypeptide having essentially the same properties of the polypeptide of the instant invention and thereby attempt to circumvent the literal scope of Applicants' patent rights based on any of the circumstances described above.

Applicants submit that the information disclosed in the specification combined with the knowledge of the art provides sufficient guidance to one of ordinary skill in the art to isolate such nucleic acids from other strains. The written description as a whole is sufficient to evidence possession of the claimed nucleic acid sequences because the claimed nucleic

acid sequences are defined by relation to the structure of the sequence of SEQ ID NO:1 as well as the inherent properties of the polypeptide encoded by the nucleic acid sequences of SEQ ID NO:1. Thus, there is sufficient written description in the specification to inform the skilled artisan that Applicants were in possession of the claimed phospholipases at the time the application was filed. However, to further prosecution, Applicants have amended the new claims now recite "at least 80% identity" and "at least 80% homology."

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112 have been overcome. Applicants respectfully request reconsideration and withdrawal of the rejections.

IV. The Rejection of Claims 51-56, 58, 59, and 61-66 under 35 U.S.C. § 112, First Paragraph

Claims 51-56, 58, 59, and 61-66 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Office Action states:

> [T]he specification, while being enabling for an isolated nucleic acids specified as SEQ ID NO:2 encoding a polypeptide having phospholipase [activity] or the nucleic acid having the sequence encoding the polypeptide of SEQ ID NO:1, is not enabling for 1.) isolating nucleic acid sequences coding for a polypeptide having phospholipase B activity wherein the nucleic acid has only 65% homology to nucleotides 568-2045 of SEQ ID NO:2, 2.) isolating nucleic acid sequences coding for polypeptides having phospholipase activity wherein the nucleic acid codes for a polypeptide having 65% homology to amino acids 20 to 646 of SEQ ID No:2, or 3.) isolating sequences with phospholipase B activity hybridizing under low stringency conditions to nucleic sequences anticipated by SEQ ID NO:2, 4.) identifying allelic variants of any phospholipase B gene hybridizing under low stringency conditions to nucleic acids sequences of SEQ ID NO:1, 5.) identifying allelic variants of any phospholipase B gene hybridizing under low stringency conditions to the nucleic acid sequences anticipated by the polypeptide of SEQ ID NO:2, and 6.) identifying nucleic acid subsequences encoding polypeptides having phospholipase B activity.

This rejection is respectfully traversed.

For the same reasons explained in section III (see above) and further reasons described below, Applicants assert that the specification enables any person skilled in the art to practice the invention commensurate in scope with the instant claims.

The Office Action states, "[c]ritical to the use of the invention commensurate with the

claims is the ability to identify genes with phospholipase B activity." The Office Action cites Agnan *et al.*, who suggest that the ideal probe contains the complete gene segment as the DNA probe. The Office Action then states that "applicants claim nucleic acids comprising only a fraction of the phospholipase B gene" as probes and concludes that "[g]iven the potentially enormous number of sequences claimed, applicants have not provided a means for predictably identifying sequences which have 65% homology or identity to the disclosed SEQ IDs which also have phospholipase activity." The Office Action further states that "applicants have not provided the design of even one hybridization probe capable of identifying a nucleic acid sequence having phospholipase activity under any sort of hybridization condition." These conclusory statements are incorrect.

Applicants describe enabling methods for conducting Southern hybridization under medium, medium-high, and high stringency conditions. Conditions for medium, medium-high, and high stringency are detailed on page 6, lines 17-28, of the specification. Applicants further describe the following probes in the specification for use in conducting the hybridization: (i) nucleotides 568 to 2045 of SEQ ID NO:1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO:1, and (iii) a complementary strand of (i) or (ii). These probes consist of the mature coding region of SEQ ID NO:1, which is the most essential part of the entire phospholipase B gene. Use of this portion of the gene enables the identification and isolation of other genes that are closely related or essentially identical to the gene of SEQ ID NO:1 encoding a polypeptide having phospholipase B activity.

Applicants also detail on page 12, line 29, to page 13, line 8, of the specification, instructions for determining the degree of identity between two amino acid sequences by the Clustal method (Thompson *et al.*, 1994, *Nucleic Acids Research* 22: 4673-4680; Thompson *et al.*, 1997, *Nucleic Acids Research* 25: 4876-4882). Once a gene is isolated and its sequence determined, the deduced amino acid sequence can then be compared to that of SEQ ID NO:2 to ascertain whether it falls within the scope of the instant claims. This is well within the skill of the art.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112 are overcome. Applicants respectfully request reconsideration and withdrawal of the rejections.

V. The Rejection of Claims 65 and 66 under 35 U.S.C. § 112, First Paragraph

Claims 65 and 66 stand rejected under 35 U.S.C. § 112, first paragraph, as containing

subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Office Action states:

> [T]he specification, while being enabling for the production of a polypeptide using techniques well known in the art of protein expression and purification, does not reasonably provide enablement for the expression and purification of said polypeptide in all strains of microorganisms. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

This rejection is respectfully traversed.

Applicants have provided detailed methods for expressing genes encoding a phospholipase and methods for recovering and purifying the phospholipases. On page 14, line 8, to page 24, line 14, of the specification, Applicants have provided enabling details for constructing recombinant expression vectors for expressing the claimed genes in recombinant host cells. In fact, Examples 5 describe the construction of an expression construct for expressing the gene of SEQ ID NO:1 in *Aspergillus oryzae*. Applicants have demonstrated the ability to express the phospholipase gene in an *Aspergillus oryzae* host cell. Once the expression of the gene has been achieved in a fungal host cell such as *Aspergillus oryzae*, one skilled in the art can predict that the gene can be expressed in other fungal host cells.. For other host cells other than of a fungal nature, one skilled in the art can then determine what regulatory sequences, vectors, selectable markers, etc. should be used to accomplish expression of the gene, using Applicants' enabling disclosure.

The Office Action also suggests that "it is not apparent that, even if in a given microorganism the gene were transcribed and a protein translated, the recovery of said polypeptide would be easily accomplished by one skilled in the art." Applicants have provided methods for producing and recovering phospholipases of the instant invention on page 27, line 6, to page 28, line 22, of the specification. Isolation and purification of the phospholipase of SEQ ID NO:2 to homogeneity is described in Example 6. Any purification protocol is based on the physical and chemical properties of the protein. Once isolation and purification methodologies have been established, they are repeatable no matter what the source of the phospholipase. Any minor modifications in the protocols are well within the ability of one skilled in the art. Thus, recovery and purification of the phospholipases from other microorganisms is predictable and well within the skill of the art.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejections.

VI. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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